

# Exploration of Morphospace Using Procrustes Analysis in Statoliths of Cuttlefish and Squid (Cephalopoda: Decabrachia)—Evolutionary Aspects of Form Disparity

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**Abstract.** This paper reports on a pilot study using, for the first time, a Procrustes type analysis of shape in exploring the morphospace of cephalopod statoliths. A total of 12 species of cuttlefish and squid (Decabrachia) from the Mediterranean were analyzed, based on 18 homologous points (landmarks) chosen on the anterior statolith surface. For two species (one cuttlefish, one loliginid squid) size ranges were sufficiently large to reveal ontogenetic trends in statolith. Comparisons between species resulted in four well-defined sets of statolith morphology corresponding, respectively, to (1) sepiid cuttlefish, (2) *Rossia* (a large sepiolid squid), (3) myopsid squids, (4) oegopsid squids and small sepiolids. The morphological "dissociation" of large and small sepiolids suggests a relation between statolith size and shape "distinctness," and draws attention to the possibly paedomorphic shapes at the lower end of the size scale.

## INTRODUCTION

Statoliths are calcareous particles that are attached to an epithelial receptor complex inside the paired statocysts of cephalopods. They grow by periodic addition of aragonite layers crystallized from the statocyst fluid. They are part of the macula/statolith system for the detection of gravity and other linear acceleration. This gravity receptor system functions along with a complementary angular acceleration receptor system, which occupies the greater part of the statocyst (Budelman et al., 1997). The statoliths have been studied intensively since the beginning of the 1960's (e.g., Young, 1960; Clarke & Maul, 1962; Lombarte et al., 1997). In recent years, much attention has been given to the growth layers observed in squid statoliths as potential age markers (Jereb et al., 1991; Arkhipkin & Bizikov, 1997; Bizikov & Arkhipkin, 1997).

Clarke & Maddock (1988a, b) came to the conclusion that the shape of the statoliths depends little on their function and heavily on the phylogeny. In contrast, the overall structure of the statocysts apparently reflects rather strongly the respective life style and locomotor activity of a species (Young, 1988, 1989). Thus the question remains open whether statolith form, even if it appears to depend little on the function of the statolith (Clarke & Maddock, 1988b), might nevertheless reflect some physical constraints related to the movement of the endolymph inside the statocyst.

Our study reconsiders the conclusion of Clarke & Maddock (1988b) as to phylogenetic information provided by statolith morphology, in (1) exploring morphospace patterns in statoliths of several decabrachian groups, considering variability at different levels from intraspecific to intergroup (family) level, (2) assessing biological form disparity between statoliths in terms of morphological distances that are testable against the phylogenetic trees derived from molecular methods, and (3) identifying likely homoplasies by comparing traditional taxonomy, exploitation of molecular patterns and statolith morphology. This approach should allow us to consider two evolutionary aspects complementary to one another, namely adaptive significance versus genetic fixation of statolith morphology.

Form disparity needs to be described in morphological terms applied to the disparity definition proposed by Raff (1996), according to which disparity is the measure of how fundamentally different organisms are. Methods of geometric morphometry (Procrustes analysis) have proved interesting on theoretical grounds (Bookstein, 1991) and efficient in their applications to various zoological groups (e.g., Tabachnik & Bookstein, 1990; Laurin et al., 1994; Neige & Dommergues, 1995; David & Laurin, 1996; Neige & Boletzky, 1997). These methods are based on the utilization of anatomically conspicuous points (landmarks *sensu* Sneath, 1967; Bookstein et al., 1985; Bookstein, 1991). A given set of landmarks serves

Table 1

Traditional classification of the studied taxa (after Mangold & Portmann, 1989), and number of individuals (n) used for analysis.

Order	Suborder	Family	Subfamily		n		
SEPIOIDEA		Sepiidae		<i>Sepia officinalis</i> Linnaeus, 1758	11		
				<i>Sepia elegans</i> d'Orbigny, 1835	11		
				<i>Sepia orbignyana</i> Férussac, 1826	11		
		Sepiolidae	Rossiinae		<i>Rossia macrosoma</i> (Delle Chiaje, 1829)	03	
				Sepiolinae		<i>Sepietta neglecta</i> Naef, 1916	05
						<i>Sepietta oweniana</i> (Pfeffer, 1908)	07
					<i>Sepiola</i> sp.	01	
TEUTHOIDEA	Myopsida	Loliginidae		<i>Loligo vulgaris</i> Lamarck, 1798	32		
				<i>Alloteuthis media</i> (Linnaeus, 1758)	31		
	Oegopsida	Enoploteuthidae		<i>Abralia veranyi</i> (Rüppell, 1844)	01		
			Ommastrephidae	Illicinae		<i>Illex coindetii</i> (Vérany, 1837)	09
							<i>Todaropsis eblanae</i> (Ball, 1844)

as a morphological descriptor. Comparison of the relative positions of these landmarks warrants localization and quantification of morphological differences between ontogenetic stages, individuals, or taxa. Such a representation is called a morphospace (Neige et al., 1997); here it is given in the form of a phenogram for cephalopod statoliths.

## MATERIALS AND METHODS

Our analysis covers nine genera: four Sepioidea, five Teuthoidea (Table 1). This sample represents a wide range of decabrachian diversity and covers the greater part of taxa studied recently with molecular methods (Bonnaud et al., 1996, 1997; Boucher-Rodoni & Bonnaud, 1996). All the specimens were caught in the area of Banyuls-sur-Mer (western Mediterranean).

### Landmarks

Although the statoliths of decabrachian cephalopods show a wide variety of forms, their structure is sufficiently constant to permit recognition of homologies. In our study, homologies are derived from a subdivision of the whole statolith in four basic compartments according to the terminology of Clarke (1978): attachment area or wing, ventral rostrum, lateral dome, and dorsal dome (Figure 1A). Nevertheless our approach of morphology using landmarks is different from the one chosen by Clarke & Maddock (1988b) who took linear measurements for subsequent processing by multivariate analysis.

In this study, only the anterior side of the statolith is analyzed. Apart from the fact that the attachment area, which is one of the most important structural elements, is entirely exposed only on the anterior side, this side has the advantage of showing a clear partitioning, which allows one to recognize a large number of homologous points (Figure 1B). Since the position of maximum sur-

face curvature in the lateral dome (Point 17) is used, our descriptor also provides some information on the relief of the anterior face. Figure 1C gives the localization and assessment of the 18 landmarks as adopted in this study, taking into account the terminology of Bookstein (1991).

### Data Acquisition and Morphometric Treatment

Homologous points, localized on camera lucida drawings of statoliths, were seized using a digitizer (3SPACE<sup>®</sup> DIGITIZER). The adjustments permitting form comparisons were achieved with the LSTRA algorithm of the Procrustes program (David & Laurin, 1992). The phenetic trees derived from a distance matrix, calculated with the Procrustes program (see Appendix 1, 2, 3), were obtained using the Fitch algorithm of the PHYLIP program (Felsenstein, 1990).

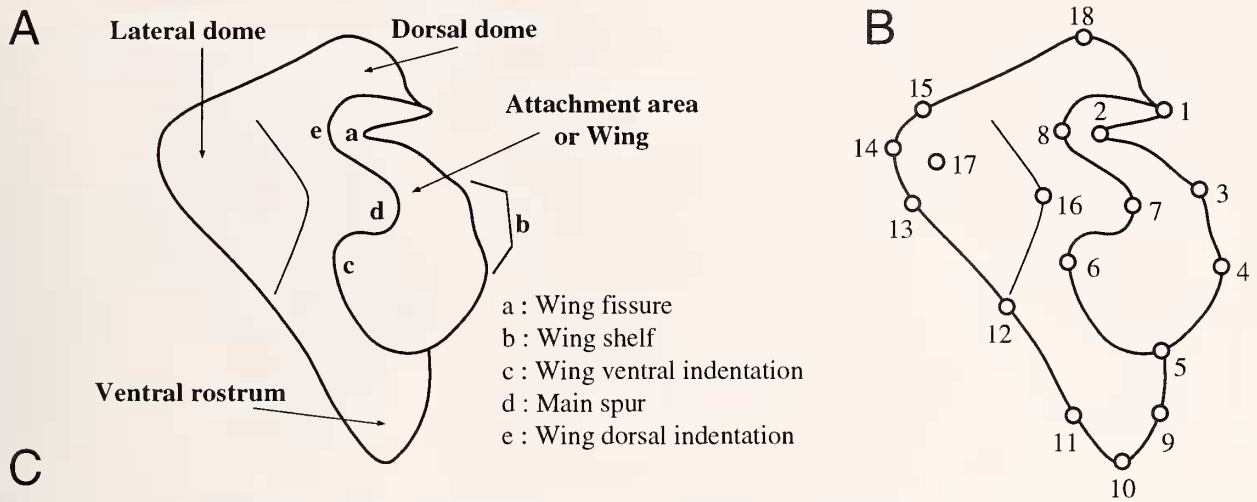
The first step of the analysis was to compare all the individuals in pairs (Pairwise analysis of Procrustes software) within each species. In some cases, this step could allow one to surmise an ontogenetic trend in statolith morphology. To test the veracity of this observation, a phenetic tree is established that summarizes all the pairwise comparisons. If such a trend is confirmed, the sample is represented for the following interspecific analysis by two categories: one for small specimens, the other for large ones.

To compare species, an average individual is calculated (Consensus Analysis option of Procrustes software) from all individuals included in the species, except in ontogenetically marked species where two average individuals are calculated: one for small specimens, the other for large ones.

## RESULTS

### Variation within Species

For *Loligo vulgaris*, if the largest individual is compared with the smallest, the adult statolith appears rela-



Number	Type	Description
1	2	Medialmost point of wing spur
2	2	Medialmost point of wing fissure
3	2-3	Dorsalmost point of wing shelf
4	2-3	Ventralmost point of wing shelf
5	1	Medial contact of ventral rostrum and wing
6	2	Lateralmost point of wing ventral indentation
7	2	Medialmost point of main spur
8	2	Lateralmost point of wing dorsal indentation
9	3	Intersection of a parallel to the axis (5-10) and rostrum medial edge
10	2	Ventralmost point of ventral rostrum
11	3	Intersection of a parallel to the axis (10-12) and ventral rostrum
12	2	Lateral contact of lateral dome and ventral rostrum
13	3	Intersection of a parallel to the axis (12-14) and lateral dome
14	2	Lateralmost point of lateral dome
15	3	Intersection of a parallel to the axis (14-18) and lateral dome
16	2	Medialmost point of lateral dome
17	2	Maximum swelling of dorsal dome
18	2	Dorsalmost point of lateral dome

Figure 1

A. Statolith morphology. B. Localization of homologous points used. C. Definitions and respective types (*sensu* Bookstein, 1991) of the 18 homologous points used.

tively wider (Figure 2). The tree constructed from the distance matrix (Appendix 1) is arranged from the smallest to the largest individuals (Figure 3). Thus the feeble morphological modification during growth is indeed a directed one. The same obtains in *Sepia officinalis*. In our sample, there is progressive shortening of the rostrum and widening of the attachment area (Appendix 2, Figure 4). Thus for the interspecific analysis *Loligo vulgaris* and *Sepia officinalis* are represented by two categories each:

SMALL (smaller than 120 mm ML in *L. vulgaris*, smaller than 130 mm ML in *S. officinalis*) versus LARGE.

The statoliths of other species analyzed under identical conditions (*Sepia elegans*, *S. orbignyana*, *Alloteuthis media*) do not reveal growth-related morphological modifications. *Rossia macrosoma*, *Sepietta neglecta*, *S. oweniana*, *Sepioloa* sp., *Abralia veranyi*, *Todaropsis eblanae*, *Illex coindetii* are not represented by sufficiently different ranges of sizes to allow an ontogenetic analysis. Never-

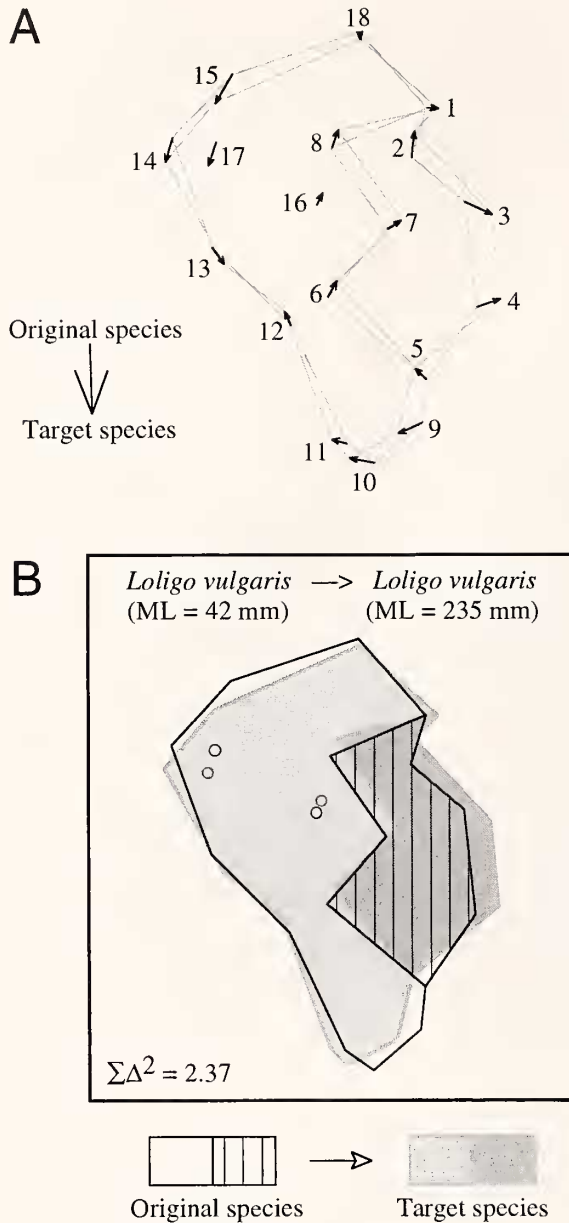


Figure 2

An example of comparison using LSTRA (comparison between two individuals of *Loligo vulgaris* of different sizes). A. Vector field resulting from Procrustes adjustment. B. Presentation used in this analysis.

theless, for *Illex coindetii*, recent data from a very wide range of sizes reveal an ontogenetic change of statolith shape (Gonzalez & Guerra, 1997). Our present data correspond to stage 2 described by these authors.

Even when sexes were distinguishable in sufficiently large numbers of individuals (*Sepia elegans*, *S. orbignyana*, *Illex coindetii*), no sexual dimorphism was detect-

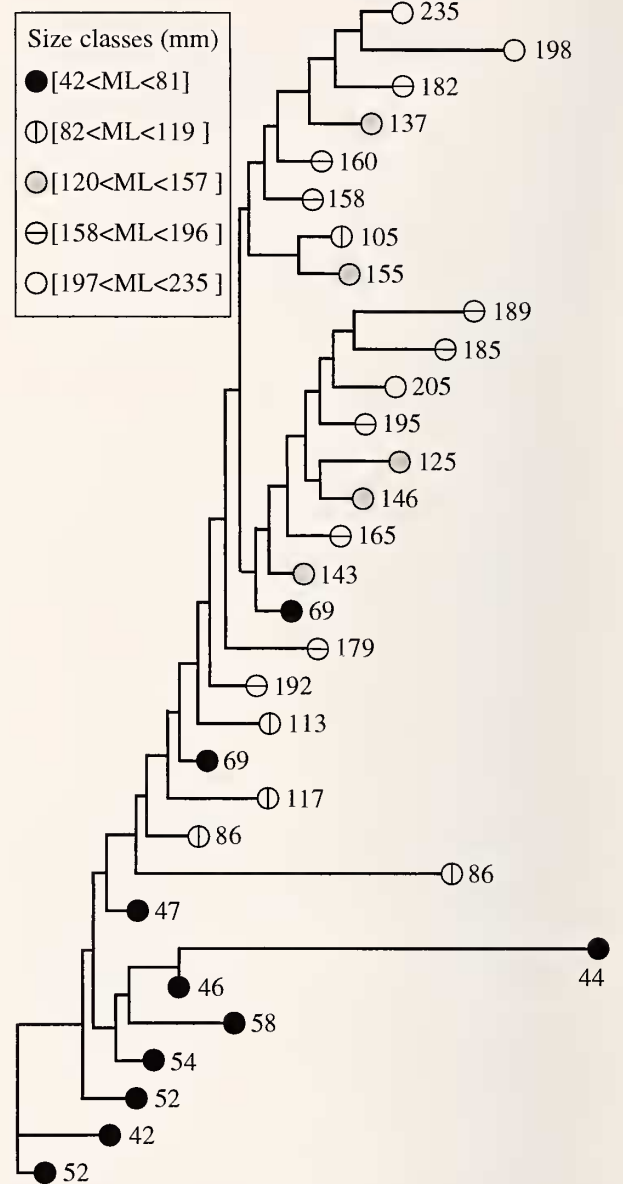


Figure 3

Phenetic tree for *Loligo vulgaris* showing the hierarchy of statolith morphologies as a function of mantle length (ML).

able in statolith morphology; males and females never clustered separately in the phenograms.

### Variation between Species

Pairwise analyses among loliginids (*Alloteuthis media* and *Loligo vulgaris*) show that statoliths of *Alloteuthis media* of any size resemble more closely the SMALL *Loligo vulgaris* than the LARGE ones (Figure 5A, B). Differences are rather modest (max.  $\Sigma\Delta^2 = 1.58$ , Appendix 3) and are expressed mainly by the overall form of

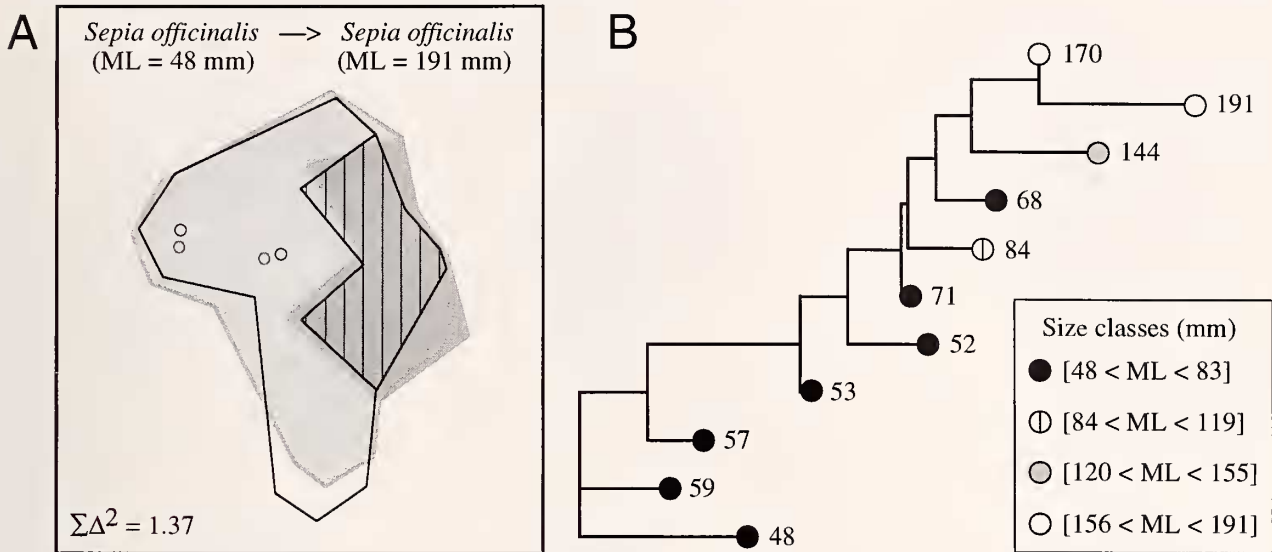


Figure 4

A. Comparison between two individuals of *Sepia officinalis* of different mantle lengths. B. Phenetic tree for *Sepia officinalis* showing the hierarchy of statolith morphologies as a function of mantle length (ML).

the statolith, which is more slender in *Alloteuthis media* and in SMALL *Loligo vulgaris*, and by the lateral and ventral expansion of the lateral dome, which is very marked in LARGE *Loligo vulgaris*. In contrast, the ventral rostrum shows only very few morphological modifications. This result suggests an ontogenetic heterochrony: *Alloteuthis media* having pedomorphic statoliths compared to *Loligo vulgaris*.

The morphological differences between oegopsid squids (*Abralia veranyi*, *Illex coindetii*, *Todaropsis eblanae*) are undeniable (max.  $\Sigma\Delta^2 = 3.38$ , Appendix 3), but they do not affect the general pattern of statolith morphology (Figure 5C): the ventral rostrum is short, the attachment area is long compared to the rest of the statolith, the overall form is slender, and the lateral dome is rather indistinct.

A comparison between the two teuthoid groups (Myopsida versus Oegopsida) reveals notable differences (for example a  $\Sigma\Delta^2$  of 3.73 between LARGE *Loligo vulgaris* and *Illex coindetii*; Figure 5D, Appendix 3). In comparison with *L. vulgaris*, *I. coindetii* has statoliths in which the rostrum is markedly reduced, the lateral dome truncated laterally, but well developed dorsally and ventrally, and which has a longer attachment area.

The three species of *Sepia* Linnaeus, 1758, have very similar statoliths (max.  $\Sigma\Delta^2 = 1.53$ , Appendix 3), always with a lateral dome forming a very distinct subspherical structure (which sets them apart from all the other taxa studied), a long and broad rostrum showing a truncated or broadly rounded end, and a large, massive attachment area (Figure 5E). The Sepiolineae form another group with high morphological coherence, although interspecies dis-

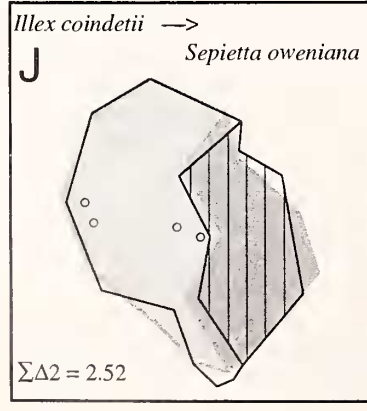
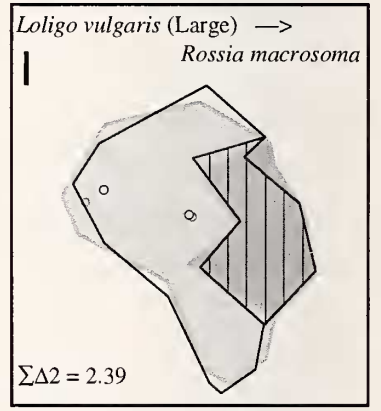
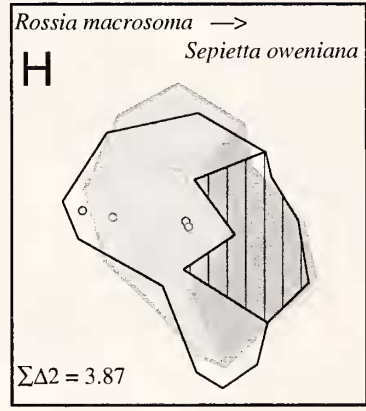
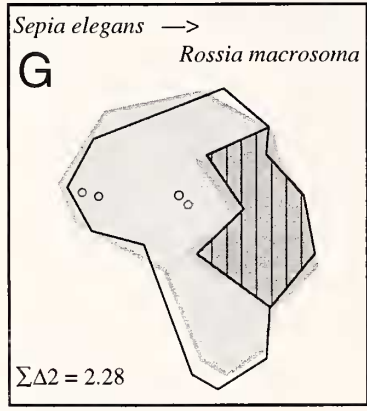
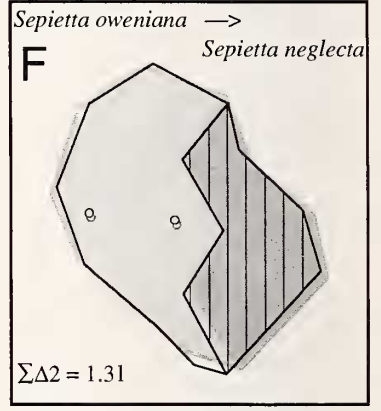
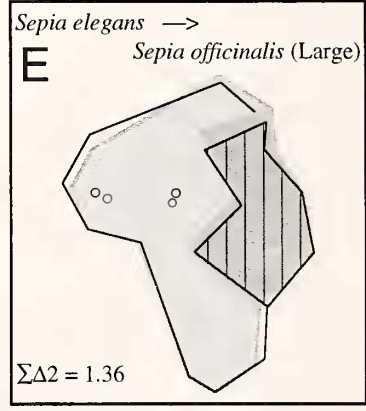
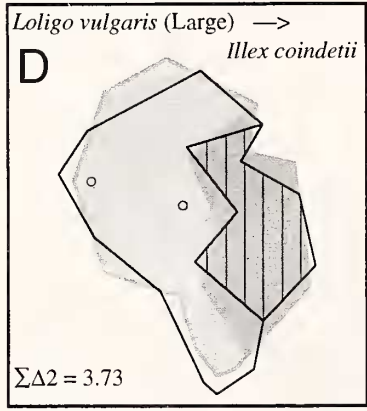
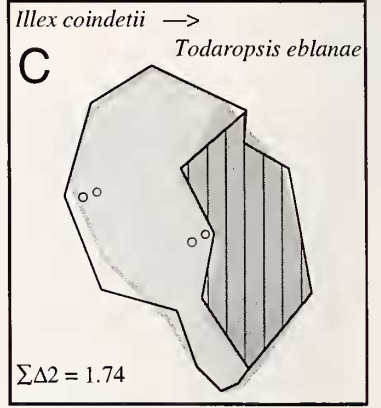
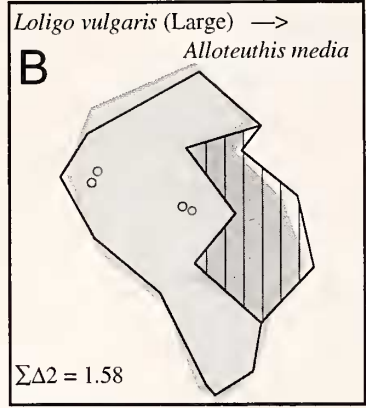
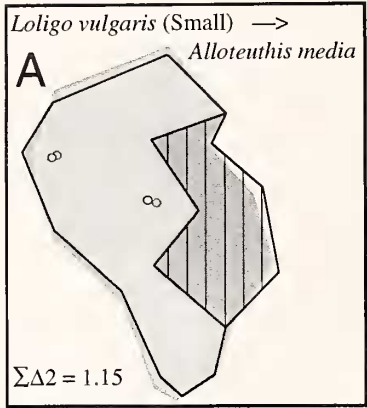
tances are larger (max.  $\Sigma\Delta^2 = 2.49$ , Appendix 3). Their statoliths are characterized by the lack of a ventral rostrum (which implies superposition of points 5, 9, 10, 11; Figure 5F), a strongly (especially dorsally) reduced attachment area, and by a well-developed dorsal dome, which is only poorly demarcated from the lateral dome, however. The overall outline is slender in the statoliths of the Sepiolineae.

The analyses reveal particularly marked differences between Sepiidae and Sepiolineae (max.  $\Sigma\Delta^2 = 5.05$ , Appendix 3). An intermediary position between these two sets is held by the sepiolid *Rossia macrosoma* (Figure 5G, H), which appears closer to the sepiids (max.  $\Sigma\Delta^2 = 3.02$ , Appendix 3) than to the Sepiolineae (max.  $\Sigma\Delta^2 = 4.43$ , Appendix 3). Apart from the lack of a (typically sepiid) subspherical lateral dome, the main difference between *Rossia macrosoma* and sepiid statoliths is the smaller rostrum with a more rounded end and a wider lateral dome.

Moreover, the pairwise analyses performed between *Sepia elegans* and *Rossia macrosoma*, on the one hand (see Figure 5G), and between *Loligo vulgaris* and *Rossia macrosoma*, on the other (Figure 5I), highlight the intermediary position of *R. macrosoma* between the Sepiidae and myopsid Teuthoidea. In contrast, the Sepiolineae appear closer to the oegopsid Teuthoidea, as shown by the comparison between *Illex coindetii* and *Sepietta oweniana* (Figure 5J).

## DISCUSSION

The phenetic tree derived from the distance matrix (Appendix 3) gives a quantitative representation of relations



between statolith morphologies in the species studied (Figure 6). It reflects many of the taxonomic divisions traditionally accepted (Voss, 1977; Mangold & Portmann, 1989; Sweeney et al., 1992).

For the Teuthoidea, the phenetic tree topology obtained with our method suggests a clear separation of Myopsida (with loliginid genera *Loligo* Lamarck, 1798, and *Alloteuthis*, Wülker, 1920) and Oegopsida (represented by two families, the Enoploteuthidae with *Abralia* Gray, 1849, and the Ommastrephidae with *Illex* Steenstrup, 1880, and *Todaropsis* Girard, 1890). In contrast, the Sepioidea do not cluster. *Sepia* and *Rossia macrosoma*, on the one hand, differ from the Sepiolinae (*Sepiolo* Leach, 1817, *Sepietta* Naef, 1912), on the other. Thus the statoliths of the Sepiolidae fall under two distinct morphologies, which correspond to the Rossiinae and Sepiolinae, respectively.

Clarke & Maddock (1988b:182) concluded that statoliths largely confirm a broad pattern as expected from general systematic studies. However, for the Sepiolidae, they note that *Heteroteuthis* and *Rossia* are closer to the Sepiidae than is *Sepiolo*.

The morphological similarity between the statoliths of *Rossia macrosoma* and *Sepia* could be related to similarities in both animal and statolith sizes between *Sepia* and *Rossia macrosoma*, but the adult size of *Heteroteuthis* Gray, 1849, is closer to that of *Sepiolo* or *Sepietta*. This has to be kept in mind in attempts to interpret the disparity between the statolith morphologies of Rossiinae and Sepiolinae in relation to functional constraints. Likewise, the morphological similarity between statoliths of Sepiolinae and of oegopsid squids could be related to similarities in statolith size, but it cannot be related to the adult sizes of the animals, which are very different. Thus the question arises whether this similarity of statolith morphology might reflect a close phylogenetic relationship. This has to be discussed in relation to other published data, and especially with a close look at *Idiosepius* Steenstrup, 1881.

Various data now call for a redefinition (or abandonment) of the higher taxa Sepioidea and Teuthoidea. Within the Teuthoidea, the suggested transfer of *Chtenopteryx* Appellöf, 1890, from the Oegopsida to the Myopsida (to join the loliginids, as suggested by Young, 1991, and Brierley et al., 1996), is a minor change, but it may foreshadow greater rearrangements. The removal from the Sepioidea, either of the Sepiolidae together with the Sepiariidae and the Idiosepiidae (Fioroni, 1981), or of the Sepiolidae with the Idiosepiidae only (Clarke, 1988), has already launched a reassessment of sepiolid relationships

(Boletzky, 1995). The resulting systematic changes within the former Sepioidea, or within the Decabrachia as a whole, are now highlighted by the possible transfer of *Idiosepius* to the Oegopsida based on nucleotide and amino acid sequences processed with the Neighbour Joining method, Bonnaud et al. (1997).

Provided that this new position of *Idiosepius* is not due to an artefact of data processing (especially relating to use of a distance method), the similarity of the statoliths of *Idiosepius* (Jackson, 1988) with those of Sepiolinae suddenly appears in new light. Indeed these statoliths are similar not only to those of Sepiolinae, but also to those of ommastrephid and enoploteuthid squids. Of course, this observation should not be taken to mean that the Sepiolinae or the whole family Sepiolidae would now have to follow in the wake of *Idiosepius* in view of the other similarities (cf. Fioroni, 1981; Clarke, 1988).

In contrast to the new position of *Idiosepius* suggested by the above-mentioned molecular study, phylogenetic relationships of the Sepiolidae change depending on which subfamilies are included in the analysis: when represented by *Sepietta* and *Heteroteuthis* the Sepiolidae cluster with the Sepiidae (Bonnaud et al., 1997), when represented by *Rossia* Owen, 1834, and *Sepiolo*, they cluster with a group containing ommastrephids along with loliginids and sepiids, the latter two forming terminal sister groups (Boucher-Rodoni & Bonnaud, 1996).

An interesting parallel appears in the distribution of morphological characters in decabrachian spermatozoa. Healy (1989, 1990) observed that the respective forms in the cuttlefish *Sepia*, in the loliginids *Loligo* and *Alloteuthis*, and in the sepiolids *Rossia* and *Sepietta* are similar for the differentiation of a mitochondrial spur at the flagellar basis. In contrast, the acrosome comes in two forms, one (rounded) showing greater similarity between sepiid cuttlefish and loliginid squids, the other (elongate) showing greater similarity between different sepiolids (including *Heteroteuthis*). However, the spermatozoa of *Heteroteuthis* are different from those of *Rossia* and *Sepietta*; they have a periflagellar mitochondrial sleeve instead of a mitochondrial spur. Surprisingly, this mitochondrial sleeve is very similar to what exists in *Spirula* Lamarck, 1799, whereas the (elongate) acrosomes of spirulid spermatozoa are in their turn similar to those of the sepiolid spermatozoa in general. But other morphological and molecular data do not suggest *Spirula* to be the closest relative of sepiolids.

In both instances, statolith morphology and spermatozoan morphology, one has to cope with a mosaic of features. This situation necessitates a careful reassessment of

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Figure 5

Some examples of pairwise comparisons selected to illustrate the morphological differences within studied Decabrachia.

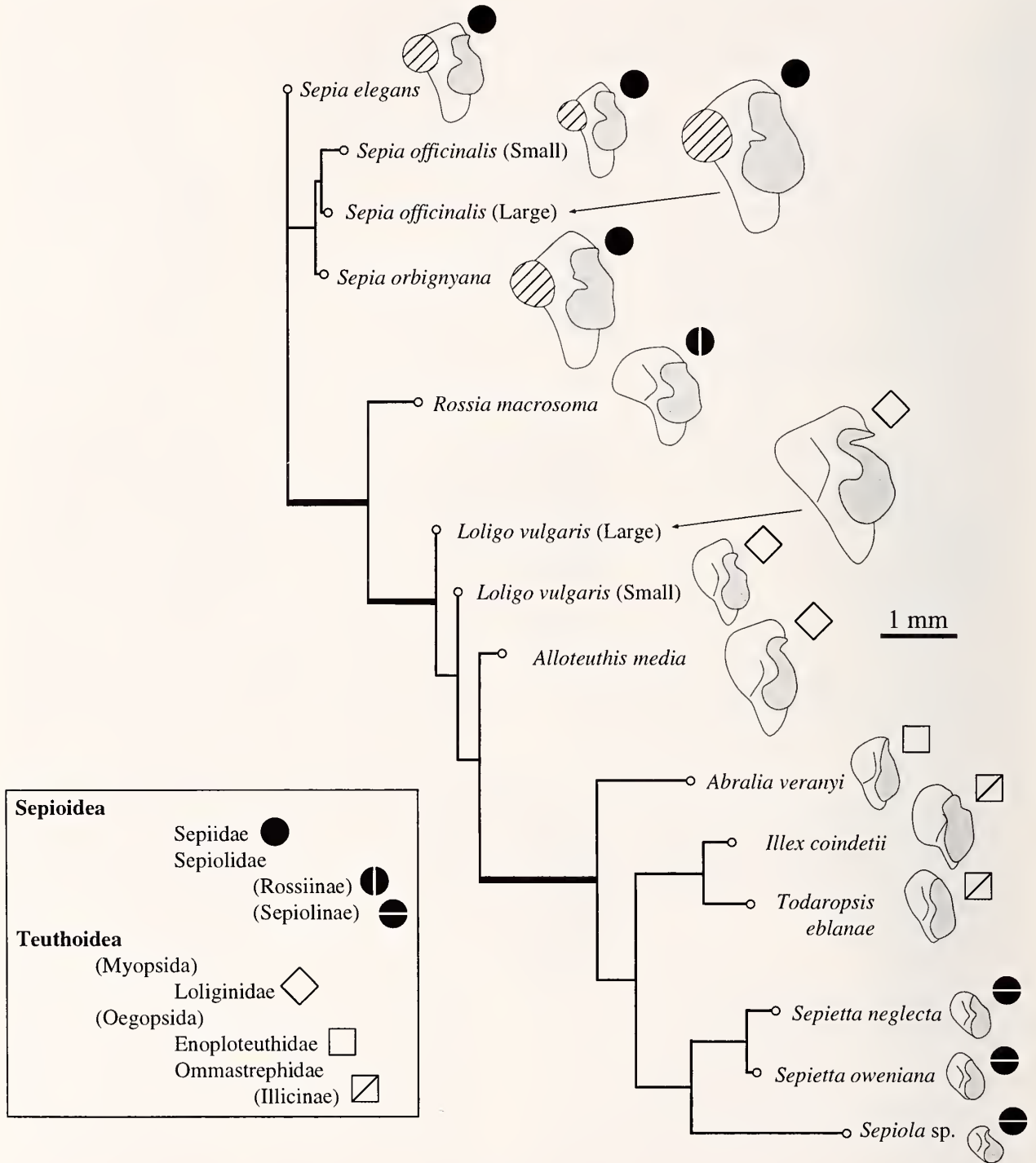


Figure 6

Phenetic tree constructed from the quantification of differences and similarities in the statoliths of 12 analyzed species. All statoliths are drawn to the same scale. Note the overall size decrease from the top to the bottom of the figure.



each of the characters considered. As to statolith morphology, the subglobular shape of the lateral wing in *Sepia* then appears as a truly distinctive feature (a likely synapomorphy) that is much more significant as a phylogenetic signature than the overall structure of the statolith. For the rest, it is the *lack* of such distinctive features that leads to an ostensible mixture of systematic groups, similarity of form being inversely related to statolith size (Figure 6). More data from the subfamily Rossiinae, especially from the small juvenile statoliths, are needed to see whether the statoliths of the Sepiolinae represent paeodomorphic forms derived from an ancestral situation closer to that of *Rossia*, or whether the latter results from hypermorphosis *sensu* Gould (1977). Complementary sets of ontogenetic data from the Heteroteuthinae might also be instructive.

Of course, statoliths have to be viewed also on the background of statocyst morphology and related functional constraints. Describing the different statocysts in the three subfamilies of Sepiolidae, Young (1989:224) noted that the statocysts are basically all alike in being the shortest among all cephalopods relative to volume. Only in *Rossia*, some emphasis on the horizontal channel occurs (supposedly related to turning in the yawing plane). Discriminant analysis separates these statocysts from those of *Sepia* and places them close to those of the non-buoyant teuthoids. Perhaps this description of the statocysts provides some insight into the peculiar form of statoliths in *Rossia*; the well-developed lateral wings might reflect the emphasis on the horizontal channel and its supposed role in monitoring turning in the yawing plane. It also highlights similarities between sepiolids and non-buoyant teuthoids such as loliginids, ommastrephids, and enoploteuthids.

In conclusion, morphological studies using Procrustes analysis do not escape the inherent limitations of phenetic clustering in phylogeny reconstruction (de Queiroz & Good, 1997). In contrast, morphospace defined by Procrustes analysis based on different ontogenetic stages provides a highly objective (reproducible) representation of specific structures through developmental time. This analysis sharpens our view for detecting trends in ontogenetic form change and for relating them to the functional integration of organ complexes. More detailed knowledge of physical (especially rheological) statocyst/statolith adjustments (by coadaptation) in different cephalopods may yield new character state definitions that are phylogenetically significant.

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Appendix 1  
Distance matrices ( $\Sigma^2$ ) for 32 individuals of *Loligo vulgaris* (#: collection numbers).

#001	#002	#005	#009	#010	#011	#017	#018	#019	#021	#022	#023	#024	#025	#027	#029	#030	#031	#041	#042	#043	#062	#077	#078	#087	#088	#089	#101	#104	#105	#110	
#002	1.55																														
#005	1.36	1.20																													
#009	1.76	1.46	1.18																												
#010	1.90	1.92	1.23	1.20																											
#011	1.54	1.19	1.10	0.92	1.45																										
#017	1.28	1.30	1.20	1.43	1.82	1.07																									
#018	1.20	1.40	1.26	1.84	1.99	1.53	1.23																								
#019	1.56	0.89	1.09	1.38	1.68	1.15	1.10	1.46																							
#021	1.57	1.43	1.30	1.50	1.55	1.24	1.25	1.29	1.42																						
#022	1.28	1.46	0.95	1.37	1.39	1.31	1.37	1.16	1.40	1.05																					
#023	1.41	1.23	1.22	1.41	1.70	1.41	1.10	1.14	1.26	1.39	1.20																				
#024	1.51	1.44	0.99	1.04	1.46	1.20	1.64	1.36	1.40	1.22	1.39	1.22																			
#025	2.14	1.94	1.57	1.41	1.27	1.44	1.67	1.93	1.68	1.69	1.72	1.40	1.62																		
#027	1.15	1.02	1.18	1.32	1.88	1.10	1.04	1.35	1.18	1.33	1.27	1.29	1.21	1.96																	
#029	1.24	1.19	0.84	1.26	1.46	1.23	1.26	1.16	1.30	1.25	0.89	0.82	1.15	1.47	1.06																
#030	1.77	1.41	1.32	1.42	1.77	1.31	1.48	1.83	1.43	1.77	1.74	1.32	1.60	1.52	1.26	1.21															
#031	1.38	1.68	1.31	1.81	1.78	1.56	1.44	1.42	1.45	1.36	1.25	1.67	1.53	2.07	1.54	1.63	1.96														
#041	2.81	2.54	2.24	2.06	2.02	2.19	2.65	2.96	2.52	2.77	2.75	2.46	2.39	1.73	2.56	2.50	2.08	2.95													
#042	1.95	1.49	1.47	1.02	1.33	1.09	1.65	2.05	1.48	1.78	1.75	1.54	1.46	1.09	1.52	1.48	1.32	1.94	1.56												
#043	2.37	2.53	1.84	1.96	1.80	2.07	2.33	2.53	2.50	2.15	1.94	2.42	1.95	2.32	2.30	2.24	2.52	1.84	2.48	2.11											
#062	2.04	1.68	1.40	1.12	1.22	1.13	1.69	1.88	1.81	1.40	1.46	1.60	1.44	1.28	1.59	1.37	1.62	1.91	2.02	1.02	1.84										
#077	1.79	1.53	1.23	1.43	1.76	1.28	1.38	1.48	1.41	1.35	1.16	1.33	1.47	1.71	1.41	1.34	1.64	1.61	2.51	1.69	2.01	1.58									
#078	1.74	1.42	1.28	1.42	1.61	1.22	1.09	1.49	1.48	1.30	1.42	1.19	1.24	1.42	1.47	1.18	1.53	1.52	2.52	1.54	2.13	1.39	1.49								
#087	1.70	1.25	1.21	1.22	1.14	0.97	1.31	1.77	1.37	1.47	1.49	1.39	1.31	1.39	1.29	1.26	1.72	1.74	0.91	2.03	0.98	1.58	1.38								
#088	2.20	2.11	1.65	1.46	1.07	1.60	2.10	2.40	1.88	2.09	2.01	2.06	1.59	1.55	1.92	1.72	2.16	1.85	1.29	2.09	1.48	1.82	2.00	1.35							
#089	2.09	1.64	1.44	1.29	1.28	1.43	1.73	2.02	1.68	1.81	1.80	1.34	1.71	1.23	1.71	1.55	1.46	2.06	1.63	0.97	1.87	1.09	1.74	1.68	1.03	1.39					
#101	1.84	1.34	1.39	1.68	2.05	1.35	1.21	1.50	1.35	1.43	1.46	1.30	1.57	1.56	1.44	1.36	1.52	1.74	2.52	1.71	2.47	1.75	1.10	1.42	1.46	2.16	1.70				
#104	1.40	1.27	1.51	1.86	2.21	1.43	1.08	1.45	1.25	1.45	1.55	1.03	1.66	1.74	1.27	1.25	1.50	1.56	2.66	1.84	2.62	2.06	1.36	1.36	1.65	2.37	1.94	1.08			
#105	1.36	1.25	1.22	1.45	1.76	1.28	1.21	1.00	1.31	1.24	1.02	1.12	1.37	1.80	0.96	1.06	1.60	1.35	2.74	1.76	2.30	1.62	1.09	1.43	1.66	2.17	1.78	1.44	1.20		
#110	1.87	1.56	1.77	2.38	2.70	1.80	1.41	1.65	1.51	2.18	2.23	1.58	2.16	1.77	1.87	1.85	2.16	2.75	2.21	3.07	2.59	2.02	1.70	2.00	2.80	2.35	1.56	1.26	1.93		
#111	1.49	1.29	0.91	1.34	1.34	1.25	1.26	1.24	1.16	1.29	1.16	1.13	1.35	1.39	1.35	0.93	1.11	1.39	2.19	1.38	2.22	1.31	1.29	1.24	1.16	1.59	1.32	1.33	1.49	1.20	1.73

## Appendix 2

Distance matrices ( $\Sigma\Delta^2$ ) for 11 individuals of *Sepia officinalis* (#: collection numbers).

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	#044	#046	#047	#048	#050	#112	#113	#114	#116	#117
#046	1.55									
#047	1.36	1.20								
#048	1.76	1.46	1.18							
#050	1.90	1.92	1.23	1.20						
#112	1.54	1.19	1.10	0.92	1.45					
#113	1.28	1.30	1.20	1.43	1.82	1.07				
#114	1.20	1.40	1.26	1.84	1.99	1.53	1.23			
#116	1.56	0.89	1.09	1.38	1.68	1.15	1.10	1.46		
#117	1.57	1.43	1.30	1.50	1.55	1.24	1.25	1.29	1.42	
#134	1.28	1.46	0.95	1.37	1.39	1.31	1.37	1.16	1.40	1.05

## Appendix 3

Distance matrices ( $\Sigma\Delta^2$ ) for 12 decabrachian species studied.

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	Amedi	Icoin	Seleg	Sorby	Sepsp	LvuIS	LvuLL	SoffS	SoffL	Rmacr	Snegl	Sowen	Todsp
Icoin	3.43												
Seleg	3.06	5.18											
Sorby	3.42	5.25	1.27										
Sepsp	4.43	4.16	4.72	5.05									
LvuIS	1.15	3.31	2.85	3.10	4.27								
LvuLL	1.58	3.73	2.11	2.44	4.31	1.01							
SoffS	3.41	5.29	1.53	1.34	5.11	3.11	2.49						
SoffL	3.63	5.49	1.36	0.98	4.82	3.33	2.68	1.11					
Rmacr	3.01	4.43	2.28	2.74	4.43	2.56	2.39	3.02	2.9				
Snegl	3.49	2.87	4.44	4.68	2.47	3.38	3.64	4.62	4.56	3.75			
Sowen	3.11	2.52	4.55	4.78	2.49	3.08	3.41	4.67	4.67	3.87	1.31		
Todsp	3.63	1.74	5.70	5.76	4.01	3.57	4.18	5.83	6.02	4.88	3.03	2.77	
Avera	3.15	3.38	4.30	4.37	4.05	3.15	3.49	4.39	4.39	4.47	3.77	3.59	3.08